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Durable Control of Metastatic AKT1-Mutant WHO Grade 1 Meningothelial Meningioma by the AKT Inhibitor, AZD5363

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Abstract: High-throughput analyses have revealed the presence of activating mutations in the AKT1 gene in a subpopulation of meningiomas. We report a female patient with multiple intracranial tumor manifestations and histologically verified meningotheliomatous meningioma in the lung. The tumor was continuously growing at multiple sites despite six surgical resections, radiotherapy, and two lines of systemic therapy. Following detection of an AKT1E17K mutation in three independent tumor samples by sequencing, treatment with AZD5363, a selective AKT inhibitor, was initiated. Ex vivo cultured meningioma cells exhibited sensitivity to the drug as shown by pAKT accumulation on immunoblots. Treatment with AZD5363 resulted, for the first time, in stable disease and minor radiographic response. The patient has been on that treatment for more than one year with ongoing clinical and radiographic response. This is the first report of an AKT1-mutant meningioma responding to AKT inhibition, suggesting that molecular screening may result in clinical benefit.

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Brief Communication

Durable control of metastatic AKT1-mutant WHO-grade I meningothelial meningioma by the AKT inhibitor, AZD5363

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Abstract

High throughput analyses have revealed the presence of activating mutations in the *AKT1* gene in a subpopulation of meningiomas. We report a female patient with multiple intracranial tumor manifestations and histologically verified meningotheliomatous meningioma in the lung. The tumor was continuously growing at multiple sites despite six surgical resections, radiotherapy and 2 lines of systemic therapy. Following detection of an AKT1^{E17K} mutation in 3 independent tumor samples by sequencing, treatment with AZD5363, a selective AKT inhibitor, was initiated. Ex vivo cultured meningioma cells exhibited sensitivity to the drug as shown by pAKT accumulation on immunoblots. Treatment with AZD5363 resulted, for the first time, in stable disease and minor radiographic response. The patient has been on that treatment for more than one year with ongoing clinical and radiographic response. This is the first report of an AKT1-mutant meningioma responding to AKT inhibition, suggesting that molecular screening may result in clinical benefit.

Meningiomas are the most common tumors of the meninges and more frequent than primary brain tumors. Recent high throughput analyses have revealed activating mutations in the *AKT1* gene in a small subpopulation of these tumors, preferentially of meningothelial subtype (1-3). Here we report a female patient diagnosed in 1995 at the age of 37 with left sphenoid wing WHO grade I meningotheliomatous meningioma (4). The patient provided written approval of this publication. Local resections were done in 1995, 1998 and 2002. In June 2014, an intraorbital manifestation was resected which corresponded to a WHO grade I meningioma. In September 2014, a left temporal tumor manifestation was partially resected and diagnosed as WHO grade III meningioma. Postoperative radiotherapy (50 Gy) was given in 1998. In 2009, a thoracic CT scan carried out for prolonged coughing showed multiple bilateral pulmonary nodules histologically verified to represent somatostatin receptor-positive metastatic meningioma (**Figure 1A-D**). Octreotide therapy was instituted, but resulted in a syndrome reminiscent of experimental allergic encephalomyelitis which we interpreted in the context of a history of multiple allergies (5).

In 2011 the patient was exposed to sorafenib, which resulted in regression of tumor-associated edema but had to be discontinued because of increased liver enzymes. A drug holiday and re-exposure to a lower dose confirmed sorafenib intolerance. In 2014 an *AKT1*^{E17K} mutation was confirmed by PCR and direct sequencing in 3 independent tumor samples obtained from 2 sites in the brain and a pulmonary meningioma manifestation (**Figure 1E**).

Ex vivo cultures from the left temporal meningioma were generated from excess surgical material from the patient using a papain dissociation system (Worthington Biomedical Corporation, Lakewood, NJ, USA) and kept in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal calf serum (FCS)

until enough cells were available for the experiments. For the experiments, the cells were kept in serum-free DMEM and exposed to increasing concentrations of AZD5363 for 72 h. Cellular lysates were assessed for phosphoAKT (pAKT), total AKT and a downstream target, phospho glycogen synthase kinase (pGSK)-3, by immunoblot (6). The following antibodies, diluted 1:1000, from Cell Signaling Technology (Boston, Massachusetts, USA) were used: rabbit anti phospho-AKT (Thr308), rabbit anti phospho-AKT (Ser473), rabbit anti-AKT and rabbit anti phospho-GSK-3β (Ser9). The membranes were exposed to HRP-conjugated secondary rabbit-specific antibodies (Sigma-Aldrich, St Louis, MO, USA). The cell cultures exhibited sensitivity to AZD5363, a selective inhibitor of the kinase activity of Akt (7), as verified by pAKT accumulation across a concentration range from 1 to 9 µM (**Figure 1F**), although with only moderate growth inhibition and no cytotoxicity at concentrations at up to 10 µM as assessed by trypan blue dye exclusion (*data not shown*).

Because of further progression at multiple sites (**Figure 2A-D**), beginning November 2014, the patient was started on AZD5363 at 480 mg twice daily in a 4 days on/3 days off regimen. Treatment was overall well tolerated, with occasional minor diarrhea, but no alterations of routine laboratory parameters including complete blood count and liver and kidney function tests, and resulted, for the first time, not only in stable disease, but a minor radiographic response (**Figure 2E,F**). The reversal of the previous growth trend under AZD5363 treatment for all meningiomas is graphically demonstrated in **Figure 2G**. Reduction in size achieved over the treatment period of 17 months varied between 12.5% (orbital meningioma), 11.1% (tentorial), and 7.0% for the left temporal meningioma.

The patient has remained on that treatment for more than one year with clinical and radiographic benefit last documented in April 2016. CT scans of the chest before initiation of AZD5363 treatment as well as 8 months later demonstrated no

signs of progression of the intrapulmonary meningioma manifestations (**Supplementary Figure 1**). This is the first documented report of an AKT1-mutant metastatic meningioma showing prolonged benefit from targeted therapy using an AKT inhibitor. Although this is just a single case observation and long-term efficacy and tolerability of AZD5363 remain to be demonstrated, molecular screening and targeted treatment of this subgroup of meningioma patients is warranted.

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Notes

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Figure Legends

Figure 1. Histological and molecular features. A. In 1995, biopsy of the sphenoid wing tumor revealed a WHO grade I meningothelial meningioma (HE, scale bar: 50 µm). B. Intraorbital meningioma with transitional features, including psammoma bodies, WHO grade I (HE, scale bar: 50 µm). C. Metastatic meningioma, grade I, within pulmonary alveoli (*) (HE, scale bar: 50 µm). D. In 2014, biopsy showed a meningioma with atypical features including sheeting and tumor cells with abundant eosinophilic cytoplasm and eccentric irregular nuclei, occasionally with discrete nucleoli (HE, scale bar: 10 µm). E. Electropherogram depicting the base exchange G>A at codon 17 with consecutive amino acid exchange E>K in AKT1. F. *Ex vivo* cultured meningioma cells were exposed to increasing concentrations of AZD5363 for 72 h. Cellular lysates were assessed for pAKT, total AKT and a down-stream target, p-glycogen synthase kinase (GSK) 3, by immunoblot.

Figure 2. Neuroimaging features. Axial and sagittal T1-weighted gadolinium-enhanced MRI (0.9 mm) on 19-Feb-2009 and 16-Oct-2014 before and 17 months after initiation of AZD5363 therapy (18-Apr-2016). **A, B**. MRI obtained in February 2009 (**A,B**) at time of pulmonary meningioma diagnosis depicts left sphenoid wing meningioma, cavernous sinus and orbital apex meningiomas, 3 left tentorial incisural meningiomas and left temporal meningioma. **C, D**. Follow-up in October 2014 shows progression of all meningiomas despite experimental treatments with octreotide and sorafenib. Residual meningiomas are present within orbital apex and at left sphenoid wing after partial resection of both orbital apex tumor 6/2014 and of left temporal meningioma. **E, F**. Following 17 months of AZD5363 treatment (4/2016), MRI indicates growth arrest and regression in size between 1 and 2.5 mm (- 7 to - 12.5%) of all meningiomas best spotted for the incisural meningiomas which become more

separable. **G**. Growth patterns of 6 intracranial meningioma manifestations. The maximum diameter of each meningioma is shown at the indicated time points. The dotted vertical line indicates the last MRI before initiation of AZD5363 treatment. Three tumors had a minor size reduction by surgery before start with AZD5363 therapy which is depicted by the interrupted curves.